Effects of 3-chloro-phenyl-1,4-dihydropyridine derivatives on *Trypanosome cruzi* epimastigotes

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Abstract

A series of 3-chloro-phenyl-1,4-dihydropyridine derivatives produced different degrees of inhibition of parasite growth and respiration on clone Brener, LQ and Tulahuen strains of *Trypanosome cruzi* epimastigotes. Respiratory chain inhibition appears to be a posible determinant of the trypanosomicidal activity of this compounds. No difference in the action of these derivatives was found among the different parasite strains. For comparative purposes, the inhibitory effects of felodipine and nicardipine are also reported. A good correlation between toxic effects and the easiness of oxidation of the dihydripyridine ring was found. The presence of a fused ring on the dihydropyridine moiety significantly diminished the inhibitory effects.

Keywords: 3-chloro-phenyl-1,4-dihydropyridine; Parasite growth; *Typanosoma cruzi*; Felodipine; Nicardipine

1. Introduction

Chagas disease (American Trypanosomiasis) is a serious health problem in Latin American countries, where over 20 million people are already infected with *Trypanosoma cruzi*, the protozoan parasite that causes this disease. Mortality indices range from 8 to 12% depending on patient's age and physiological state (WHO Expert Commitee, 1991).

Two nitroheterocylic drugs, nifurtimox and benznidazole, have been used to treat this disease, but the serious side effects produced in 40–70% of the patients force half of them to stop treatment. Furthermore, important differences in susceptibility to these drugs have been detected among the many different parasite strains isolated (Filardi and Brener, 1987; Gustafsson et al., 1987; WHO Expert Commitee, 1991; Morello et al., 1994; Maya et al., 1997). Nifurtimox is no longer being used in some countries because of its toxicity and

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its ineffectiveness in chronic stages of Chagas' disease. Due to the inadequacy of the available chemotherapeutic regimens, hundreds of chemical compounds – both natural and synthetic – have been tested as antichagasic agents, but the potential of actual toxicity and the low water solubility of many of these have curtailed their use (Chiari et al., 1991; Sepúlveda-Boza and Cassels, 1996; Rivas et al., 1999).

Among the tested compounds, a series of nitro aryl 1,4 dihydropyridine derivatives proved to be active against several strains of *T. cruzi* epimastigotes (Núñez-Vergara et al., 1997). The results demonstrated that all compounds have an inhibitory effect on growth and respiration of the parasites. In the present communication, we extend the studies to other series of 3-chloro-phenyl-1,4-dihydropyridine derivatives. Also, for comparative purposes, we have included two well-known 1,4-dihydropyridines drugs, such as, felodipine and nicardipine.

2. Materials and methods

2.1. Chemicals and drugs

Tryptose, fetal calf serum, yeast extract and tryptone were obtained from Difco. Hemin and all other chemicals were purchased from Sigma Chemical Co.

Nicardipine and Felodipine were obtained from Chile Laboratories, Santiago, Chile.

2.2. 3-chloro-phenyl-1,4-dihydropyridines

Compound I to V (Fig. 1) synthesis was reported previously (San Feliciano et al., 1993; Caballero et al., 1996). They were performed following a modified Hantzsch procedure, through condensation of the convenient unsaturated ketoesters and enamines.

2.3. Parasites

T. cruzi epimastigotes (Brener clone, Tulahuén and LQ strains), from our collection, were grown at 28°C in Diamond's monophasic medium as reported earlier (Aldunate et al., 1986), with blood replaced by 4 μM hemin. Fetal calf serum was added to a final concentration of 4%.

2.4. Inhibition of culture growth

Drugs were added in dimethylsulfoxide (DMSO) solutions, to reach the final concentrations in the culture medium indicated in the tables and figures. *T. cruzi* epimastigote growth was followed by nephelometry using culture flasks with a side arm (Ferreira et al., 1988; Aldunate et al., 1992).

2.5. Oxygen uptake

Respiration measurements were carried out polarographically with a Clark No 5331 electrode (Yellow Spring Instruments) in a Gilson 5/6 oxygraph (Letelier et al., 1990). The chamber volume was 2 ml and the temperature was 28°C. The amount of parasites used for the assays was equivalent to 2 mg of protein. The parasites were resuspended in 0.05 M potassium phosphate buffer, pH 7.4, containing 0.107 M sodium chloride. Drugs were added at a 100 μM final concentration in DMSO. Control respiration was 35 ± 5 n-at. oxygen/min per mg of protein. Values are expressed as the mean $\pm$ SD of three or more independent experiments.

2.6. Drug toxicity

To perform toxicity determinations, parasite suspensions (3×10^6 cells/ml clone Brener in Diamond media) were incubated for 2 and 24 h at 28°C, with drugs at a 100 μM concentration in DMSO. Changes in parasite motility and shape were microscopically ($40 \times$ magnification) observed (Letelier et al., 1990).

Toxicity grades expressed as 0, 1, 2, 3 and 4, represent the sequential changes in motility, shape and lysis of parasites (Letelier et al., 1990).

No effect on cell growth, oxygen consumption or drug toxicity attributable to DMSO, was observed at the maximum concentration used of 2%.

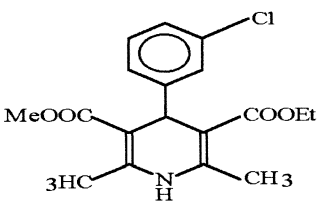
2.7. Electrochemical measurements

All the studies were carried out in aprotic media (dimethylformamide containing 0.1 M tetrabutylammonium perchlorate) using an electrochemical BAS equippment model 50W. A glassy carbon electrode, an Ag/AgCl electrode and a platinum wire electrode were used as a

working electrode, reference electrode and as auxiliary electrode, respectively. Consequently, oxidation peak potential values were measured against an Ag/AgCl reference electrode (Núñez-Vergara et al., 1997; Núñez-Vergara et al., in press).

2.8. Statistical analysis

Pearson's correlation and lineal regression analysis were performed using Prism Graphpad software from Graphpad Software INC.



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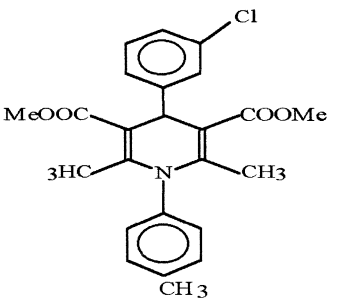

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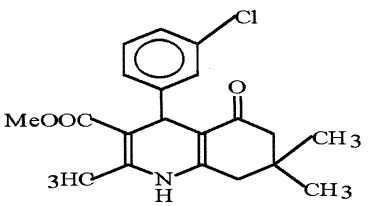

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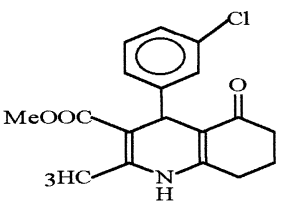

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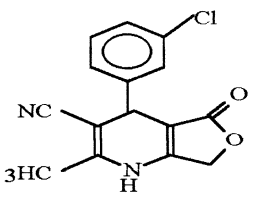

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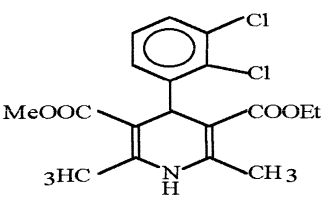
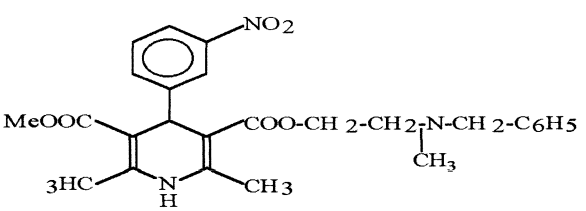


FELODIPINE



NICARDIPINE

Fig. 1. Chemical structures and names of 3-chlorophenyl 1,4-dihydropyridine derivatives (I) ethyl 4-(3-chlorophenyl)-5-methoxy-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate. (II) dimethyl 4-(3-chlolophenyl)-2,6-dimethyl-1-phenyl-1,4-dihydropyridine-3,5-dicarboxylate. (III) methyl 4-(3-chlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate. (IV) methyl 4-(3-chlorophenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate. (5) 4-(3-chlorophenyl)-2-methyl-5-oxo-1,4,5,7-tetrahydrofuro[3,4,*b*]pyridine-3-carbonitrile.

Table 1
Effect of 3-chloro-phenyl-1,4-dihydropyridines on culture growth, oxygen uptake of *Trypanosoma cruzi* epimastigotes and their correlations with oxidation peak potential values

Compound	% Inhibition culture growth[a]		% Inhibition oxygen uptake[b]	Ep, mV[c]
	10 μM	100 μM		
I	40 ± 8	80 ± 2	66 ± 3	1131 ± 5
II	38 ± 3	62 ± 6	52 ± 4	1195 ± 4
III	10 ± 3	40 ± 4	52 ± 5	1197 ± 7
IV	no inhibition	no inhibition	41 ± 4	1164 ± 6
V	12 ± 5	35 ± 3	20 ± 1	1324 ± 5
Nicardipine	40 ± 4	98 ± 2	92 ± 6	1007 ± 5
Felodipine	32 ± 6	74 ± 3	61 ± 4	1180 ± 6

[a] Calculated respect to control at the 10th day of culture growth.
[b] Calculated respect to control (35 ± 5 n-at. oxygen/min per mg protein) at a 100 μM drug concentration at 28°C.
[c] Ep: Anodic peak potential values measured in dimethylformide, expressed in millivolt. All values are expresed as the mean ± SD of three or more independent experiments. For further details see Section 3 and Fig. 2.

3. Results and discussion

Table 1 shows the effect of all the tested 1,4-dihydropyridines, including the established drugs felodipine and nicardipine, on the growth of epimastigotes of clone Brener of *T. cruzi* epimastigotes at 10 and 100 μM concentrations. The most effective compounds inhibiting growth at 10 μM concentration were compounds I, II and nicardipine. Felodipine, which structurally is very closely related to compound I, exhibited similar activity at this concentration.

Table 1 also shows the inhibition of respiration by the same derivatives at 100 μM concentration. The most active compounds are compound I, felodipine and nicardipine. The results of Table 1 indicated a reasonable good correlation between parasite growth and inhibition of respiration (correlation coefficient 0.9093, $P = 0.012$), indicating that the respiratory chain and consequently the production of ATP is an important target of the active compounds (Fig. 2B).

Table 2 shows the results of toxicity measured as changes in motility shape and lysis induced by the 3-chloro-phenyl-1,4-dihydropyridine derivatives at 2 and 24 h. The results agree reasonable well with those of Table 1, indicating that the changes observed are mainly due to the inhibition of the respiratory chain of the parasites and their energy production.

To compare strain susceptibilities, studies with Tulahuen and LQ strains of *T. cruzi* were conducted. Similar results as above reported in Tables 1 and 2 were obtained (data not shown). These studies are consistent with previous work where the drugs showing inhibition of the respiratory chain also showed no important differences among different strains of *T. cruzi* (Aldunate et al., 1986; Ferreira et al., 1988; Aldunate et al., 1992).

To obtain information on the chemical basis supporting the biological effects of the 3-chloro-phenyl-1,4-dihydropyridines on the parasite, correlations between such effects and the oxidation easiness were analyzed. As can be seen from Table 1 and Fig. 2A, all the 3-chloro-phenyl derivatives, but compound IV, show a good inverse correlation between the oxidation peak potential value and their inhibitory effects on *T. cruzi* epimastigotes at 100 μM, but not at 10 μM concentration (correlation coefficient for oxygen uptake inhibition and culture growth inhibition were -0.9916 and -0.9004, respectively ($P < 0.05$)). However, because lack of inhibitory activity over culture growth, compound IV was excluded from this analysis. Thus, the most active compound I had the lowest oxidation peak potential value. Consistently with the above results, nicardipine the most effective derivative on the inhibition of culture growth, also had the lowest oxidation potential value. A inverse correlation between Ep and the biological effects is apparent from Fig. 2A. The measured peak potential values account for the following oxidation reaction:

This oxidation pathway is similar to that occuring in vivo. Thus, 1,4-dihydropyridine derivatives undergo rapid and extensive hepatic oxidative metabolization by cytochrome P_{450} enzymes, also giving rise to pyridine analogous. Recently

(Núñez-Vergara et al., in press), we have studied by cyclic voltammetry and ESR techniques the oxidation mechanism of a new series of 4-methyl 1,4-dihydropyridines. Results from that work are in agreement with the oxidation mechanism here involved. Furthermore, other authors have reported (Tsuhako et al., 1991) that *T. cruzi* showed microsomal cytochrome reductase P_{450} activity as illustrated by its ability to reduce nitroheterocyclic drugs, such as nifurtimox and benznidazol.

An interesting fact, which is found from our results, is that the substitution of the nitrogen of the dihydropyridine ring (Fig. 1) such as in compound II had no dramatic effect on the easiness of oxidation compared with the other studied derivatives. In contrast, the presence of a fused ring on the dihydropyridine moiety seems to be relevant, as shown by the lack of activity of compound IV. Thus, 3-chloro-phenyl-1,4-dihydropyridines with a fused ring (compound III and IV) exhibited

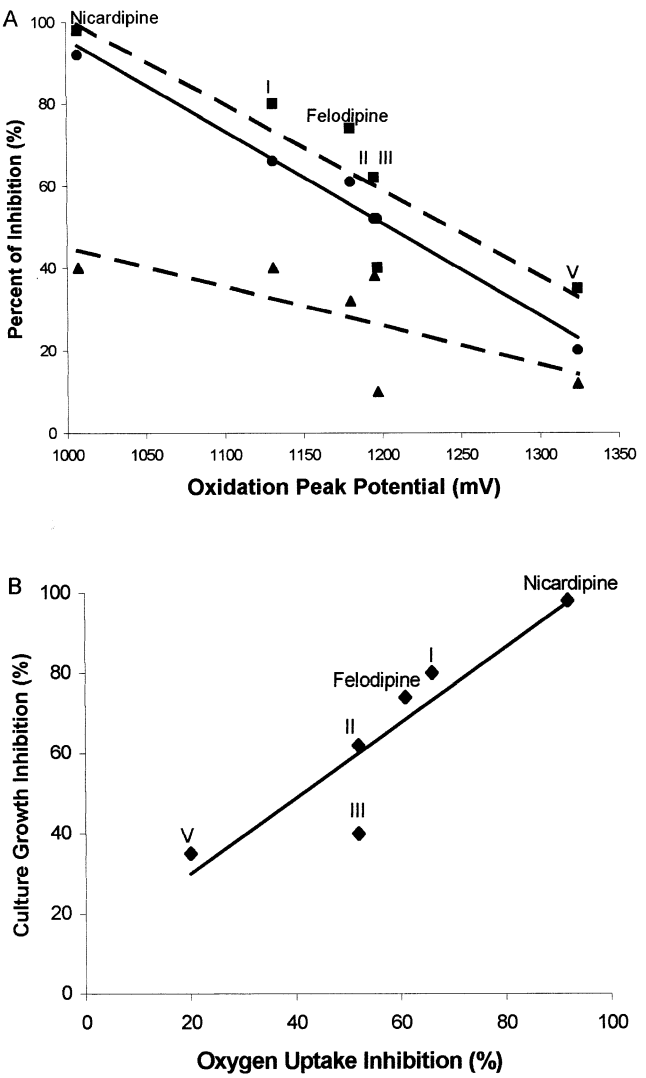


Fig. 2. (A) Relation between oxidation peak potential of 3-chloro-phenyl-1,4-dihydropyridines and *T. cruzi* epimastigotes culture growth and oxygen uptake. Oxygen uptake inhibition (closed circles and continuous line) Pearson's correlation and lineal regression coefficients are -0.9916 and 0.9832, respectively; ($P = 0.0001$). Culture growth inhibition Pearson's correlation and lineal regression coefficients at 100 μM concentration (closed squares and dashed lines) are -0.9004 and 0.8106, respectively; ($P = 0.014$) and at 10 μM concentration (closed triangles and dashed lines) are -0.7047 and 0.4967, respectively; ($P = 0.1179$). Data obtained from Table 1. (B) Correlation between oxygen uptake inhibition and culture growth inhibition of 3-chloro-phenyl-1,4-dihydropyridines on *T. cruzi* Epimastigotes. Pearson's correlation and regression coefficients are 0.9093 and 0.8267, respectively ($P = 0.012$). Data obtained from Table 1.

Table 2
Toxicity of 3-chloro phenyl 1,4-dihydropyridine derivatives towards *T. cruzi* epimastigotes[a]

Compound	2 h	24 h
I	1	4
II	1	4
III	1	4
IV	0	3
V	0	1
Nicardipine	1	4
Felodipine	1	4

[a] Toxicity grades expressed as 0 (Control), 1, 2, 3, 4 represent the sequential changes in motility, shape and lysis of parasites at a 100 μM drug concentration. See Section 3.

lower inhibitory effects than those lacking of a fused ring (compound I and II).

We conclude that some of 3-chloro-phenyl-1,4-dihydropyridine derivatives exhibit significant inhibitory effects on culture growth and oxygen uptake, as well as toxic effects described by changes in shape, motility or lysis on clone Brener. Also, these effects were evidenced in Tulahuen and LQ strains (data not shown). From the results the following tentative order of activity at 100 μM concentration can be established: I > II > III > V > IV. Apparently, the presence of a fused ring on the dihydropyridine moiety produced a diminished inhibitory effect. On the other hand, a good inverse correlation between the easie of oxidation, given by the oxidation peak potential values corresponding to the oxidation of the dihydropyridine ring to the pyridine derivatives, and toxic effects was found.

Acknowledgements

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